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SCREENING OF FUNGI FROM WHEAT SEEDS

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ABSTRACT

In the experiment, the screening of fungi from wheat seeds during Oct. to Dec. 2013-2014. The results were obtained various fungi *viz. A. niger, A. flavus, A. niger, Penicillium spp., A. fumigates, P. oxalicum, A. Candidus, P. griseofulvum, Mucor spp., P. oxalicum, C. lunata, A. alternate,* and *A. ochraceus,* from eight varieties i.e. HUW-468, WH – 147, Malavashree, Gomati, Prasad, Malveey-234, UP-1109 and R.R.-21 consisted of three separate sites Market seed, Govt. seed storage and Farmer seed recorded in Allahabad district. The results were obtained various fungi from eight varieties *viz. A. niger, A. flavus, Penicillium spp., A. fumigates, P. oxalicum, A. candidus, P. griseofulvum, F. oxysporum, A. alternate,* and *F. moniliforme* from eight varieties *viz.* HUW-468, WH – 147, Malavashree, Gomati, Prasad, Malveey-234, UP-1109 and R.R.-21 consisted of three separate sites Market seed, Govt. seed storage and Farmer seed in recorded in Allahabad district. *Mucor spp.* was not present in wheat of Varanasi.

KEYWORDS: Screening, Fungi, Seed and Wheat

INTRODUCTION

Seed is the most important input for crop production. Pathogen free healthy seed is urgently needed for desired plant populations and good harvest. Many plant pathogens are seed-borne, among all seed borne pathogens; fungal pathogens hold more economic importance than bacterial and nematode pathogens (Joshi *et al*., 1986; Kulkarni and Naragund, 1986; Mathur and Cunfer, 1993; Sarri, 1986; Sharma *et al.,* 1998).

Detection of theses pathogens is based primarily on conventional methods viz., direct inspection of dry seeds, washing test, soaking test, incubation tests, blotter tests, embryo count test and filter and centrifuge extraction technique (Castro *et al.,* 1994). These methods had been in regular practice till last decades of 20th century. Nucleic acid based molecular approaches (Chahal and Pannu, 1997; Lopez *et al*., 2003), which are specific, rapid and reliable, were developed for accurate and rapid detection of seed borne pathogens.

Stored seeds are regarded as vehicle for plant pathogens over long distances (Agarwal and Sinclair, 1996). Most prevalent pathogens attacking wheat are fungi probably ranked only second to insects as cause of seed deterioration (Christensen and Kaufmann, 1965; D'Mello et al., 1993) and make the wheat grains unacceptable as food and feed (PARC, 1989). Appro-ximately 10 to 15 species of Aspergillus, Penicillium, Fusarium and Alternaria have been reported as important contaminants of cereal grains (Kroiakova et al., 1989; Adisa, 1994; Weidenboner et al., 1996; Klyszejko et al., 2005).

The present research work was designed to compare the fungi and to identify the most prevalent fungus on freshly harvested wheat grains and during three and six month's storage period. The seeds were also assessed for their nutritional

value when freshly harvested and during storage conditions.

MATERIALS AND METHODS

The present studies were carried out at Bhargava Agricultural Botany laboratory, Department of Botany University of Allahabad, Allahabad**,** during Oct to Dec 2013-2014 for the screening of fungi from wheat seeds.

Collection of Seed Samples

Seed samples were collected from different grain markets, seed corporations, companies and farmer's seed lots from two districts Allahabad and Varanasi for the screening of fungi from wheat seed. 8 varieties have been selected, each variety 10 seeds taken in the experiment. The collected samples were put into sterile polythene bags and seal properly. Seeds were disinfected with chlorox 1% for 1-2 minutes and then washed three times with distilled water (Mittal et al., 1999). Then these were properly labelled, kept in polythene bags and stored for further studies in a freezer at 10 °C until mycological testing and other processing Fernandez et al., 1985. Samples were brought into laboratory for further processing as per the methods described by Fente et al. 2001, Sekar et al. 2008.

Agar Plate Method

Agar plate method (Malone, and Muskett 1941, Neergard 1973, Agrawal 1976). Blotter paper method (ISTA, 1985) as suggested by International Seed Testing Association (ISTA 2005) was used for the detection of fungi. And isolation, seeds were incubated on Agar plates (Annonymous, 1976). Potato dextrose agar (PDA) was used in this method for the isolation of mycoflora. 20 seeds per plate were inoculated and incubated at 22 ± 2 ^{0}C. After 7 days incubated seeds were examined under steriobinocular microscope for fungi and then the isolated mycoflora were sub-cultured by single spore technique for macro and microscopic studies.

Identification of Fungi from Wheat Seeds

Fungal morphology was studied microscopically by observing colony features (Colour and Texture) and microscopically by staining with lacto-phenol, cotton blue and observe under compound microscope for the conidia, conidiophores and arrangement of spores (Aneja *et.al.,* 2001). These samples were identified on the basis of colony characteristics and microscopic examinations. Standard books and research papers were consulted during the examination of these fungi (Aneja, 2004; Rifai, 1969; Barnet and Hunter, 1999). Identification of fungi was done at 7 days. The Petri dishes were brought to the examination area in the laboratory, where each seed was examined under a microscope for growth habits of the various fungi growing in the Petri plates. Slide preparations of the various fruiting structures of the fungi were made and identified under the stereo zoom compound microscope. Seed-borne fungi, in the form of fungal colonies, were identified (Barnett and Hunter, 1972; Booth 1971; Ellis, 1971; Nirenberg, 1976; Nelson *et al*., 1983; Raper and Fennel, 1965) and counted directly under a stereomicroscope. In case of multiple fungal growths, colonies were isolated from a single wheat grain, recorded and pure cultures were maintained on PDA slants. The fungi were identified with the help of keys, monographs and text provided by several authors Barnett and Hunter, 1972; Pedro et al., 2009.

Screening of Fungi from Seed

Screening program consisted of three separate sites market seed, govt. seed storage and farmer Seed in Allahabad and Varanasi. After each screening stage the ineffective isolates were excluded from further testing. Eight varieties have been selected for the screening seed tests. The fungal isolates tested were cultured on PDA plates (diameter 85 mm) for 3–

4 weeks at room temperature.

RESULTS AND DISCUSSIONS

In the experiment, the screening of fungi from wheat seeds during Oct to Dec 2013-2014. The results were obtained various fungi *viz. A. niger, A. flavus, A. niger, Penicillium spp., A. fumigates, P. oxalicum, A. Candidus, P. griseofulvum, Mucor spp., P. oxalicum, C. lunata, A. alternate,* and *A. ochraceus*, from eight varieties i.e. HUW-468, WH – 147, Malavashree, Gomati, Prasad, Malveey-234, UP-1109 and R.R.-21 consisted of three separate sites Market seed, Govt. seed storage and Farmer seed recorded in Allahabad district. (Table 1)

Table 1: Screening of Fungi from Wheat Seed, Collected from Three Different Sites Market Seed, Govt. Seed Storage and Farmer Seed in Allahabad

Sr. No.	Verity	Market Seed	Govt. Seed Storage	Farmer Seed
1	**HUW-468**	*A.niger*, *A. flavus*	*Penicillium spp.*, *A. flavus*	*A. flavus*, *A. niger*
2	**WH - 147**	*A.niger*, *A. flavus*	*A. fumigates*, *A. flavus*	*A.niger*, *P. oxalicum*
3	**Malavashree**	*A. fumigates*, *A.niger*	*A. candidus*, *Penicillium spp.*	*A. fumigates*, *P. oxalicum*
4	**Gomati**	*P. griseofulvum*, *A. fumigatus*	*A. fumigates*, *P. griseofulvum*	*Mucor spp.*, *Penicillium spp.*
5	**Prasad**	*A.niger*, *F. oxysporum*	*F. oxysporum*, *P. oxalicum*	*Penicillium spp.*, *A. flavus*
6	**Malveey-234**	*A. flavus A. Alternate*, *C. lunata*	*A. niger*, *A. Alternate*	*A. niger*, *C. lunata*
7	**UP-1109**	*A. niger*, *A. ochraceus*	*A. niger*, *A. Ochraceus*, *A.candidus*	*A. fumigates*, *A. niger*
8	**R.R.-21**	*A. fumigates*, *A. niger*	*A. flavus*, *A. fumigatus*	*A. flavus*, *A. fumigatus*

The two *spp.* was recorded i.e. *A. niger* and *A. flavus* in Market seed, *Penicillium spp., A. flavus* was recorded in Govt. seed storage and *A. flavus, A. niger* was recorded in Farmer seed from HUW-468 variety. The two *spp.* was recorded i.e. *A.niger* and *A. flavusin* Market seed, *A. fumigates, A. flavus* was recorded in Govt. seed storage, *A. niger, P. oxalicum* was recorded in Farmer seed from WH – 147 variety. *A. fumigates, A. niger* was recorded in Market seed, *A. candidus, Penicillium spp*. was recorded in Govt. seed storage, *A. fumigates, P. oxalicum* was recorded in Farmer seed from Malavashree. *P. griseofulvum, A. fumigatus* was recorded in Market seed, A. *fumigates, P. griseofulvum* was recorded in Govt. seed storage, *Mucor spp.*, and *Penicillium spp.* was recorded in farmer seed from Gomati verity. The two *spp*. was recorded i.e. *A.niger, F. oxysporum* in Market seed, *F. oxysporum, P. oxalicum* was recorded in Govt. seed storage and *Penicillium spp., A. flavus* was recorded in farmer seed from Prasad variety. The three *spp*. was recorded i.e. *A. flavus, A. alternate, C. lunata,* in Market seed, *A. niger, A. alternata* was recorded in Govt. seed storage, *A. niger, C. lunata* was recorded in Farmer seed from Malveey-234 variety. *A. niger, A. ochraceus* was recorded in Market seed, *A. niger, A. ochraceus,* and *A. candidus* was recorded in Govt. seed storage, *A. fumigates, A. niger* was recorded in farmer seed from UP-1109 variety. The two *spp*. was recorded i.e. *A. fumigates, A. niger* in Market seed, *A. flavus, A. fumigates* was recorded in Govt seed storage and *A. flavus, A. fumigates* was recorded in Farmer seed from R.R.-21 verity in Varanasi district.

The results were obtained various fungi *viz. A.niger, A. flavus, Penicillium spp., A. fumigates, P. oxalicum, A. candidus, P. griseofulvum, F. oxysporum, A. alternate,* and *F. moniliforme* from eight varieties *viz.* HUW-468, WH – 147, Malavashree, Gomati, Prasad, Malveey-234, UP-1109 and R.R.-21 consisted of three separate sites Market seed, Govt. seed storage and Farmer seed recorded in Allahabad district. *Mucor spp.* was not present in wheat of Varanasi. (Table 2)

Table 2: Screening of Fungi from Wheat Seed, Collected from Two Different Sites Market Seed, Govt. Seed Storage and Farmer Seed in Varanasi

Sr. No.	Verity	Market Seed	Govt. Seed Storage	Farmer Seed
1	**HUW-468**	*A.niger, A. flavus*	*Penicillium spp., A. flavus*	*A. flavus, A. fumigates , A. niger*
2	**WH - 147**	*A.niger, A. flavus*	*Penicillium spp., A. fumigates, A. flavus*	*A. niger, P. oxalicum*
3	**Malavashree**	*A. fumigatus*	*A. candidus, A. fumigatus*	*A. fumigates, A. candidus*
4	**Gomati**	*Penicillium spp., A. fumigatus*	*A. fumigates, P. griseofulvum*	*Penicillium spp.*
5	**Prasad**	*A. niger, F. oxysporum*	*A. niger, A. flavus*	*Penicillium spp., A. flavus*
6	**Malveey-234**	*A. flavus, A. alternate*	*A. niger, A. alternata*	*A. niger, F. moniliforme*
7	**UP-1109**	*A. candidus*	*A. niger, A. candidus*	*A. fumigates, A. niger*
8	**R.R.-21**	*A. fumigates, A. niger.*	*A. flavus, A. fumigates, Penicillium spp*	*A. flavus, A. fumigatus*

The two spp. also was recorded i.e. *A. niger* and *A. flavus* in Market seed, *Penicillium spp*., *A. flavus* was recorded in Govt. seed storage and three *spp.* were i.e. *A. flavus, A. fumigates, A. niger* was recorded in Farmer seed from HUW-468 variety. The two *spp*. was recorded i.e. *A.niger* and *A. flavus* in Market seed, three *spp*. i.e. *Penicillium spp*., *A. fumigates, A. flavus* was recorded in Govt. seed storage, *A. niger, P. oxalicum* was recorded in Farmer seed from WH – 147 variety. *A. fumigates* was recorded in Market seed, *A. candidus, A. fumigates* was recorded in Govt. seed storage, *A. candidus, A. fumigates*, also was recorded in Farmer seed from Malavashree. The two *spp.* i.e. *Penicillium spp., A. fumigates* was recorded in Market seed, *A. fumigates, P. griseofulvum* was recorded in Govt. seed storage, *Penicillium spp.* was recorded in Farmer seed from Gomati variety. The two *spp.* also was recorded i.e. *A.niger, F. oxysporum* in Market seed, *A. niger, A. flavus* was recorded in Govt. seed storage and *Penicillium spp., A. flavus* also was recorded in Farmer seed from Prasad variety. The two *spp*. was recorded i.e. *A. flavus, A. alternate* in Market seed, *A. niger, A. alternata* also was recorded in Govt. seed storage, *A. niger, F. moniliforme* was recorded in Farmer seed from Malveey-234 variety. *A. candidus* was recorded in Market seed, *A.niger, A. candidus* was recorded in Govt. seed storage, *A. fumigates, A. niger* also was recorded in Farmer seed from UP-1109 variety. The two *spp*. was recorded i.e. *A. fumigates, A. niger* in Market seed, three *spp*. i.e. *A. flavus, A. fumigates, Penicillium spp.* was recorded in Govt seed storage and *A. flavus, A. fumigates* also was recorded in Farmer seed from R.R.-21 variety in Varanasi district.

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REFERENCES

1. Adisa A (1994). Mycoflora of post–harvest maize and wheat grains and the implication of contamination by molds. *Mol. Nutr. Food Res*., 38: 318-326.
2. Agarwal VK and JB Sinclair. L 1996. Principles of Pathology.2nd edi, CRC Press, lnc., Boca Raton, Fl. 539 pp.
3. Agarwal, V.K (1976) Techniques for the detection of seed borne fungi. *Seed Research*, 4 : 24-31.
4. Aneja KR (2004). Experiments in Microbiology, Plant Pathology and Biotechnology. Fourth edition, *New International (P) limited publishers*, India.121-128.
5. Aneja,K.R., Experiments in Microbiology, Pant pathology and Biotechnology, *Newage International Publishers*, 2001, Vol 4:157-162.
6. Anonymous. 1993. International rules for seed testing. Seed Science & Technol., 21:1-288.
7. Barnet HL, BB Hunter (1999). Illustrated genera of imperfect fungi. *The American Psychopathological society*, U.S.A.
8. Barnett HL, Hunter BB (1972). Illustrated Genera of Imperfect Fungi. Burgess Pub. Co., Minneapolis, Minnesota, p. 241.
9. Booth C (1971). The Genus *Fusarium.* Comonw. Mycol. Inst., Kew, Surrey, England, p. 237.
10. Castro C, Schaad NW, Bonde MR (1994) A technique for extracting *Tilletia indica* teliospores from contaminated wheat seeds. *Seed Sci and Technol* 22: 91-98.
11. Chahal SS, Pannu PPS (1997) Detection of *Tilletia indica* in Wheat and T. Barclayana in Rice Samples and its Implication for Seed Certification. In: Hutchins JD, Reeves JC (eds) Seed Health Testing, CAB International Pp: 153-158.
12. Christensen CM, Kaufmann HH (1965). Deterioration of stored grains by fungi. Ann. Rev. *Phytopath*., 3: 69-84
13. D'Mello JPF, Macdonald AMC, Cochrane MP (1993). A preliminary study of the potential for mycotoxin production in barley grain. *Asp. Appl. Biol*., 36: 375-382.
14. Ellis MB (1971). Demotiaceous Hyphomycetes. C. M. I., Kew Surrey England, p. 608.
15. Fente C.A., J. Jaimez Ordaz, B.I. Vazques, C.M. Franco and A. Cepeda (2001): New addivive for culture media for rapid identification of Aflatoxin-producing *Aspergillus* strains, Applied and Environment Microbiology, 21: 4858-4862.
16. ISTA. 1985. International Seed Rules for Seed Testing Seed Science and Technology 21: (Supp.) 289p.
17. ISTA, 2005, Seed Health Testing Methods and the Germination Test. In. International Rules for Seed Testing. Pub. by *Intl. Seed Test.Assoc*. Bassersdorf, Switzerland.
18. Joshi LM, Singh DV, Srivastava KD (1986) Wheat and wheat diseases in India. In: Problems and Progress of

Wheat Pathology in South Asia. Pp: 11-19. *Malhotra Publishing House*, New Delhi

19. Klyszejko A, Kubus Z, Zakowska Z (2005). Mycological analysis of cereal samples and screening of *Fusarium* strains ability to form deoxynivalenole (DON) and zearalenone (ZEA) mycotoxins. *Pol. J. Microbiol*., 54: 21–25.
20. Kroiakova S, Yang F, Boltianskaia EV (1989). Detection of toxigenic *Fusarium* strains, producing T–2 toxin, in wheat grain mycoflora by microbiologic assay. *Vopr. Pitan*., 2: 54–57.
21. Kulkarni S, Nargund VB (1986) Soil borne diseases of wheat. In: Joshi LM, Singh DV, Srivastava KD (Eds) Problems and progress of wheat pathology in South Asia. Pp: 216-229. *Malhotra Publishing House*, New Delhi.
22. Lopez MM, Bertolini E, Olmos A, Caruso P, Gorris MT, Llop P, Penyalver R, Cambra M (2003) Innovative tools for detection of plant pathogenic viruses and bacteria. *Int. Microbiol* 6: 233–243
23. Malone, J. P. and Muskett, A. E. (1997). Seed-borne fungi. Description of 77 fungus species. Sheppard, J.W. (Ed.), 19–20. International Seed Testing Association, Zurich, Switzerland.
24. Mathur SM, Cunfer BM (1993) Seed borne diseases and seed health testing of wheat. Danish Government Institute of Seed Pathology for Developing Countries. Heller up, Denmark, pp: 168.
25. Mittal, V., P. Kumar, M. Tsiros. (1999). Attribute-level Performance, Satisfaction and Behavioural Intentions over Time: A Consumption-System Approach. *Journal of Marketing* 63(2) 88-101.
26. Neergard P. (1973). Detection of seed borne pathogens by culture tests. *Seed Sci. and Technol*. 1:217-254.
27. Nelson PE, Toussoun TA, Marasas WFO (1983). *Fusarium species*. An Illustrated Manual of Identification. The Pennsylvania State Univ. *Press, Univ. Park, Pennsylvania*, p. 203.
28. Nirenberg H (1976). Untersuchungen uber die morphologische und biologischi Differenzierung in der *Fusarium* Sektion Liseeola. Mitteilungen aus der Biologischen Bundesant Salt purlandund Forstwirtschaft, Berlin-dehlena., 169: 1-117.
29. Pakistan Agricultural Research Council (1989). "Wheat Research and Development in Pakistan", Islamabad.
30. Pedro, W.C., J.M. Verkley, J.Z. Groenewald and R.A. Samson. 2009. Fungal Biodiversity. Pub. *Co. CBSKNAW* Fungal. Biodiversity centre utrecht, The Netherlands.
31. Raper KE, Fennel DI (1965). The genus *Aspergillus.* The Williams and Wilkins Company, Baltimore, p. 686.
32. Rifai M.A (1969) Revision of the genus *Fusarium* and *Alternaria.* Mycological papers 116:40-95.
33. Saari EE (1986) Wheat diseases in South East Asia. In: Joshi LM., Singh DV, Srivastava KD (Eds) Problems and progress of wheat pathology in South Asia. *Malhotra Publishing House*, New Delhi. pp: 72.
34. Sekar P, N. Yumnam, and K. Ponmurugan (2008) Screening and Characterization of mycotoxin producing fungi from dried fruits and grains. *Advanced Biotech*. 11:46-48.
35. Sharma AK, Kumar J, Nagarajan S (1998) Disease management strategy in wheat. *Indian Farmg* 18: 52-53.
36. Weidenboner M, Berleth M, Kramer J Kunzeb B (1996). Investigation about the mycoflora of selected samples of the German cereal crop 1994. *Adv. Food Sci.,* 18: 103-106.

37. Fernandez, A., R. L. Stroshine, and J. Tuite, 1985: Mold growth and carbodioxide production during storage of high-moisture corn. *Cereal Chem*. 62: 137-44.